Developing New Antiviral Agents for Influenza Treatment: What Does the Future Hold?

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Antiviral agents for the treatment of influenza are urgently needed to circumvent the limitations of current drugs in several critical areas: high frequencies of resistance to M2 inhibitors among currently circulating strains and variable frequencies of resistance to oseltamivir among A(H1N1) strains, limited efficacy of treatment and treatment-emergent antiviral resistance in cases of avian influenza A(H5N1) illness in humans, and lack of parenteral agents for seriously ill patients. Two neuraminidase inhibitors (NAIs), zanamivir and peramivir, have undergone or are undergoing clinical trials for use by intravenous or intramuscular administration, and one long-acting NAI, designated CS-8958, is under study for use by inhalation. Advances in understanding the mechanisms involved in influenza virus replication have revealed a number of potential targets that might be exploited in the development of new agents. Among these agents are T-705, a polymerase inhibitor, and DAS181, an attachment inhibitor. Combination therapy with currently available agents is supported by data from animal models but has received limited clinical study to date.

Three principal factors drive the medical need for the development of new antiviral agents for the treatment of influenza: antiviral resistance; limited antiviral efficacy in severe cases of influenza, including in influenza A(H5N1) disease; and a lack of parenteral agents. Since 2003, the frequency of viral resistance to the M2 ion-channel inhibitors (i.e., M2 inhibitors, or adamantanes)—namely, amantadine (e.g., Symmetrel; Endo Laboratories) and rimantadine (e.g., Flumadine; Forest Laboratories)—has increased rapidly among seasonal influenza A(H3N2) viruses and is now so widespread that this class of drugs has been rendered mostly ineffective, although they retain activity against most influenza A(H1N1) viruses [1, 2]. The 2007–2008 influenza season also was notable for the community circulation of influenza A(H1N1) viruses resistant to oseltamivir in many countries [3]. Emergence of resistance to M2 inhibitors and to neuraminidase inhibitors (NAIs) also has been a clinical problem in some highly immunocompromised hosts. Administration of the oral NAI oseltamivir appears to be beneficial in the treatment of seasonal influenza in hospitalized patients [4–6] and of some cases of highly pathogenic avian influenza A(H5N1) in humans, but it has not reduced the overall case-fatality rate to below ~50% for avian influenza A(H5N1) [7]. Multiple factors likely contribute to this high mortality, including late presentation for care, emergence of resistance to oseltamivir, and possibly reduced bioavailability in some patients. In this regard, and of particular relevance in the clinical management of seriously ill, hospitalized patients, no parenteral agents for the treatment of influenza are currently available. Each of these factors contributes to the need for alternative antiviral treatments, particularly with regard to pandemic preparedness, and for the consideration of combination therapy. Several of the agents that are undergoing clinical testing will be highlighted in this article.

M2-INHIBITOR RESISTANCE

Emergence of resistance during the therapeutic use of M2 inhibitors is well known; estimates of the prevalence of M2-inhibitor resistance that occurs during thera-

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Table 1. Detection of antiviral resistant influenza during treatment.

Patient group	Oseltamivir		M2 inhibitor	
	Frequency of resistance, %	Influenza virus strain(s) [reference]	Frequency of resistance, %	Influenza virus strain(s) [reference]
Outpatient				
Adults	0.4	A [8]	~30	A(H3N2) [9, 10]
Children	5.5	A [11]	27	A(H3N2) [12]
Inpatient and outpatient children	16–18	A(H1N1) [13], A(H3N2) [14]	80	A(H1N1), A(H3N2) [15]
Immunocompromised	…[a]	A(H3N2), B [16]	>33	A [17]

[a] Antiviral resistance detected in individual patients, but frequency not reported.

peutic use are shown in table 1 [9, 10, 12, 15, 17]. In addition, in recent years, the prevalence of resistance to M2 inhibitors among community A(H3N2) isolates has increased dramatically and spread globally, as shown in figure 1 [1, 2]. In 2006, this led to changes in the US Advisory Committee on Immunization Practices recommendations regarding the use of this entire class of antiviral drugs [18]. Earlier surveillance studies found that primary resistance occurred at low frequencies among seasonal isolates and that, historically, the frequency of resistance was 1%–3%. Increasing frequencies of M2-inhibitor resistance first appeared among influenza virus A(H3N2) isolates from China and Hong Kong during the 2003 influenza season and then spread globally, with rates during the 2005–2006 influenza season of >90% not only in Asia but also in the United States and of nearly 50% in Europe. Data from the 2006–2007 influenza season showed rates of M2-inhibitor resistance of >80% in Asia and the United States and >50% in parts of Europe. Very high frequencies of resistant A(H3N2) viruses continued to be detected during the 2007–2008 influenza season in the northern hemisphere. A geographically variable, increased frequency of resistance among A(H1N1) viruses also has been observed, although the overall proportion of resistant A(H1N1) viruses is not as high as that for A(H3N2) viruses.

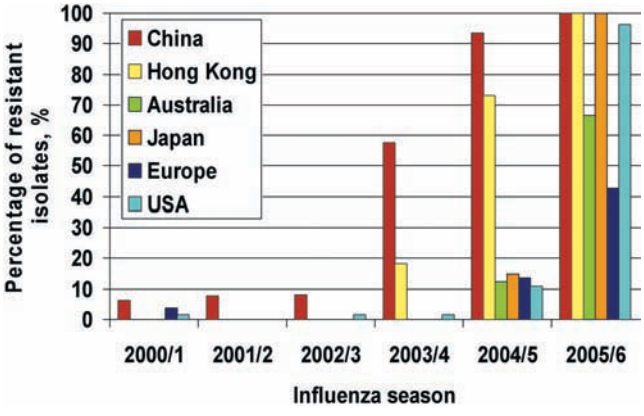

Figure 1. Antiviral resistance to M2 inhibitors in community isolates of influenza A(H3N2) virus, 2000–2006. Adapted from [1], with permission from Elsevier Ltd., and [2].

Resistance in recent A(H3N2) and A(H1N1) virus isolates has been due to a serine-to-asparagine substitution at position 31 in the M2 protein. Unfortunately, resistance to either of the current M2 inhibitors, amantadine or rimantadine, confers resistance to the entire class of M2 inhibitors, although M2 inhibitor–resistant viruses are still susceptible to the NAIs. From a public health perspective, these variants retain their virulence and their transmissibility from person to person. No loss of biological fitness has been ascertained in the laboratory or in epidemiologic studies.

Resistance to the M2-inhibitor class of antiviral drugs is also seen in many A(H5N1) viruses [19]. The clade 1 viruses that first appeared in Vietnam, Thailand, and Cambodia also are resistant because of a serine-to-asparagine substitution at position 31, the same mutation found in the resistant A(H3N2) viruses. A high frequency of M2-inhibitor resistance also has been seen in clade 2.1 viruses that circulate in Indonesia, because of this mutation or of one at position 27. On the other hand, most (>90%) of the clade 2.2 viruses that have spread across Eurasia to Europe and Africa and clade 2.3 viruses have retained sensitivity to the M2 inhibitors. Limited data suggest that amantadine was effective in some individual patients with M2 inhibitor–susceptible A(H5N1) disease in Hong Kong in 1997, when these viruses were first found to cause illness in humans [20].

NAI RESISTANCE

Resistance to NAIs occurs during therapeutic use (table 1) [8, 11, 13, 14, 16, 21] and at low frequencies among community isolates [22–24]. New mutations associated with reduced susceptibility continue to be recognized, as more-detailed surveillance is used. However, the inhibitory activity of NAIs against all 9 neuraminidase subtypes recognized in avian viruses predicts that NAIs would likely be active against a pandemic strain whether it is an A(H5N1) virus or some other novel virus that has gained its hemagglutinin and neuraminidase from an animal virus. Within the NAI class of antiviral drugs, there is

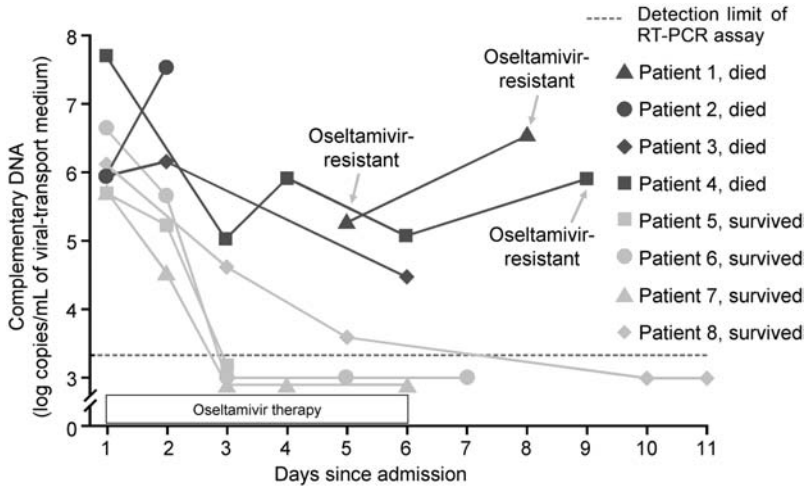

Figure 2. Influenza A(H5N1) viral load in throat swab specimens from 8 patients. Adapted from [26], with permission from the Massachusetts Medical Society.

an interesting phenomenon of variable cross-resistance that depends on the neuraminidase type and subtype, the drug, and the particular neuraminidase mutation, because of different interactions of the drugs within the active enzyme site. The practical consequence is that zanamivir (Relenza; GlaxoSmithKline) retains full inhibitory activity for several neuraminidase subtypes when mutations that confer resistance to oseltamivir (Tamiflu; Roche Laboratories) are present.

Resistance to oseltamivir also has been documented in outpatient adults, outpatient children, and inpatient children (table 1) [8, 11, 14, 16, 21]. The frequency of resistance to oseltamivir is much lower than that observed for M2 inhibitors but is not insignificant, particularly among children; resistant variants were detected in nearly 1 in 5 pediatric patients in one study conducted in Japan [1]. Not surprisingly, the frequency with which resistant virus is detected in children is substantially higher than that for adults because, in general, children have higher viral replication loads and a correspondingly greater opportunity for the emergence of resistant variants. NAI-resistant variants usually have reduced infectivity and virulence in animal models of influenza but not always. Some oseltamivir-resistance mutations are associated with full replication competence and transmissibility in animal models. Earlier studies of community isolates found that viruses with resistance mutations (based on N2 numbering) in influenza A (arginine to lysine at 292, glutamic acid to valine at 119 in the N2 neuraminidase, and histidine to tyrosine at 274 in the N1 neuraminidase) and B neuraminidases (aspartic acid to asparagine at 198, isoleucine to threonine at 222, and serine to glycine at 250) probably have been transmitted from person to person [23, 24]. Influenza A(H1N1) viruses resistant to oseltamivir because of the histidine-to-tyrosine mutation at position 274 appeared in many countries for the first time during the 2007–2008 influenza season [3]. This phenomenon has occurred in the apparent absence of selective drug pressure and indicates that these resistant variants are efficiently transmitted from person to person and capable of causing typical influenza [25].

Emergence of resistance has been documented in influenza A(H5N1) virus–infected patients treated with oseltamivir. In a case-series study of 8 patients from Vietnam, de Jong et al. [26] (figure 2) observed that a standard course of oseltamivir therapy begun a median of 6 days after the onset of illness was associated temporally with reductions in pharyngeal viral loads in half the patients and that pharyngeal viral RNA clearance at the end of the treatment course was linked with survival. In contrast, those patients who had increasing viral loads over time or failure to

Table 2. In vitro neuraminidase inhibition by peramivir, oseltamivir carboxylate, and zanamivir.

Influenza virus strain	No. of isolates	IC_{50}, median nmol/L (range)		
		Peramivir	Oseltamivir carboxylate	Zanamivir
A(H1N1)	5	0.34 (0.26–0.43)	0.45 (0.45–0.60)	0.95 (0.73–1.05)
A(H3N2)	6	0.60 (0.47–0.87)	0.37 (0.27–0.45)	2.34 (1.85–3.13)
B	8	1.36 (1.08–1.95)	8.50 (5.33–18.33)	2.70 (2.00–3.10)

NOTE. Adapted from [34], with permission from the American Society for Microbiology.

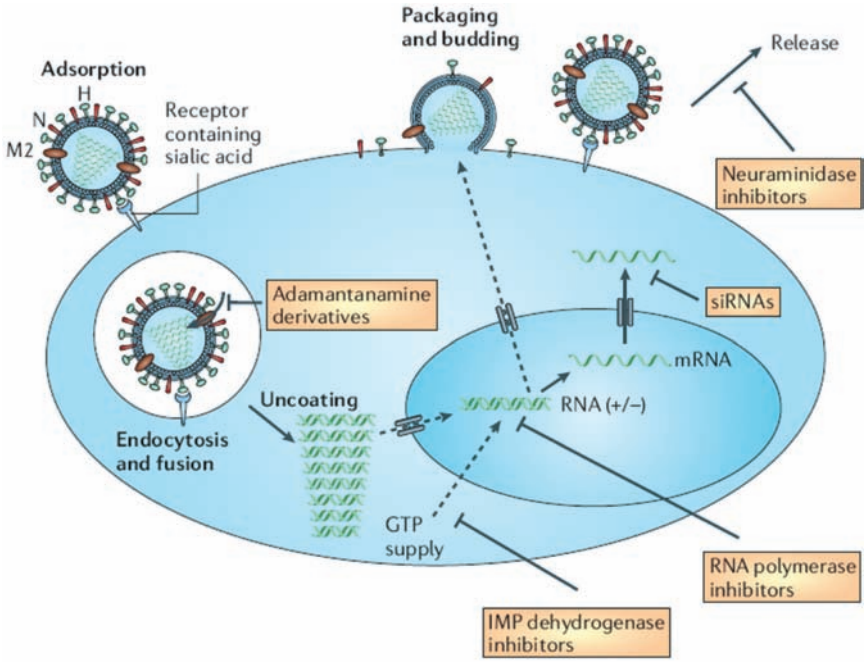

Figure 3. Influenza virus replication and sites for antiviral inhibition. GTP, guanosine 5′-triphosphate; IMP, inosine 5′-monophosphate; mRNA, messenger RNA; siRNA, small interfering RNA. Reprinted from [42], with permission from Nature Publishing Group.

clear virus succumbed to their illness. Two of these individuals had treatment-emergent oseltamivir-resistant viral variants with a histidine-to-tyrosine mutation at position 274, the most common mutation observed in N1 neuraminidase–containing viruses. N1 neuraminidase with this mutation shows marked reductions (>400-fold) in susceptibility to oseltamivir but remains inhibited by zanamivir [27, 28].

Oseltamivir treatment and prophylaxis has been used with apparent benefit in immunocompromised hosts with influenza virus infection [29–33]. However, emergence of oseltamivir-resistant variants has occurred in some immunocompromised hosts and appears to be correlated with protracted viral replication and poor clinical outcomes [16]. In some highly immunocompromised hosts, sequential therapy with both antiviral drug classes has been used, in part because viral clearance failed in these patients. Unfortunately, in this population, dual resistance to both M2 inhibitors and NAIs has been found [16]. The approach of sequential drug use in the management of influenza should be avoided for immunocompromised hosts.

LIMITATIONS OF CURRENT ANTIVIRAL TREATMENTS FOR INFLUENZA

The efficacy of current antiviral treatments is limited or uncertain in certain populations and situations. For example, oseltamivir carboxylate, the active metabolite of oseltamivir, is substantially less active against influenza B than against influenza A neuraminidases (table 2). This finding has been corroborated in several studies from Japan, which have indicated that among children, particularly young children, the treatment of influenza B with oseltamivir is associated with delayed antiviral effects and clinical resolution, compared with the treat-

Table 3. Selected investigational antiviral agents in clinical development for the treatment of influenza.

Antiviral agent	Target	Sponsor	Administration route	Phase of development
Zanamivir	NA	GlaxoSmithKline	iv	1, 2a
Peramivir	NA	BioCryst Pharmaceuticals	iv, im	2
CS-8958 (R-118958)	NA (long acting)	Sankyo Pharmaceuticals, Biota Holdings	Inhaled	2
T-705	Polymerase	Toyama Chemical	Oral	2
DAS181	HA receptor	Nexbio	Inhaled	1

NOTE. HA, hemagglutinin; im, intramuscular; iv, intravenous; NA, neuraminidase.

Table 4. Peramivir or oseltamivir improves survival of mice infected with influenza A/Vietnam/1203/04(H5N1) virus.

Compound, days of treatment	Dosage, mg/kg/day	Administration route	No. of mice			
			Survival at 15 days	Development of encephalitis	Paralysis at 15 days	Survival predicted at 15 days[a]
Peramivir[b]						
1	30	im	7	2	1	8
5	30	im	8	3	0	6
Oseltamivir, 5[b]	10	po	7	5	2	6
Saline, 1[c]	…	im	4	3	1	1

NOTE. im, intramuscular; po, by mouth. Adapted from [51], with permission from American Society for Microbiology.

[a] On the basis of a simulated death model of body-weight loss of 20%.

[b] $N = 10$.

[c] $N = 11$.

ment of influenza A [35–37]. Furthermore, no controlled studies have been completed in high-risk or hospitalized populations, although emerging evidence suggests that even delayed treatment is beneficial for adults hospitalized with serious seasonal influenza [4–6].

The efficacy of oral oseltamivir is limited in A(H5N1) disease, for which overall mortality is ~60% [38]. The case-fatality ratio among those receiving oseltamivir has hovered around 50%, compared with nearly 90% among those not receiving antiviral treatment [7]. Multiple contributing factors are likely, particularly delayed time from illness onset to antiviral treatment, and early administration (within 3–4 days after illness onset) has been associated with reduced mortality. The World Health Organization (WHO) updated its clinical management advice for avian influenza A(H5N1) virus infection in humans in 2006 and again in 2007 [39, 40]. The updated guidelines are based in part on clinical reports shared at a meeting in Turkey in March 2007 [41], and they address both antiviral treatment and supportive care and discuss the theoretical use of immunomodulators. These guidelines provide guidance on what appears to help (i.e., oseltamivir, oxygen therapy, and lung-protective ventilatory-support strategies) and on what does not (including antibiotic prophylaxis, high-dose corticosteroids, and M2-inhibitor monotherapy, especially when the virus strain is resistant to M2 inhibitors). In fact, use of corticosteroids appears to be associated with an increased risk of mortality [7].

NEW ANTIVIRAL AGENTS FOR INFLUENZA TREATMENT

Multiple potential targets are now being explored actively in the search for new antiviral treatments for influenza. Some of the potential sites of intervention are shown in figure 3 [42]. Several recent review articles have discussed comprehensively the state of the science with regard to both targets for new antiviral drug development and the status of some of the antiviral agents, particularly those in preclinical and early clinical development [42–44]. Of the many antiviral agents now in various stages of development, some agents and formulations that have shown preclinical activity and that are now being tested in human trials are listed in table 3.

Parenteral NAIs. Current antiviral treatments are given either orally or by inhalation. These routes may not provide rapid, reliable drug delivery in seriously ill patients. For example, failure of zanamivir therapy for the treatment of pneumonia in a bone-marrow transplant recipient has been reported, even though the influenza A(H1N1) virus with which the patient was infected was sensitive to zanamivir [45]. In addition, the oral bioavailability of oseltamivir, especially when given by nonstandard means (e.g., via nasogastric tube), is uncertain, although a recent report on 3 patients found adequate absorption under such circumstances [46]. Parenteral administration would circumvent these limitations by guaranteeing rapid delivery and high levels in blood to increase the likelihood of drug delivery to sites of infection, especially in those with pneumonia or, for patients with influenza A(H5N1) virus infection, extrapulmonary infection.

The poor oral bioavailability of zanamivir (~2%) is well documented [47] and provides the impetus for the development of alternate administration routes for the drug. Although an inhaled formulation is approved, intravenous zanamivir has been evaluated in phase 2a testing [48]. In healthy volunteers receiving 600 mg zanamivir or placebo intravenously twice daily for 5 days prior to a virus challenge, zanamivir was found to be highly protective against experimental infection (14% vs. 100%, respectively, were infected), to reduce viral shedding (0% vs. 100%, respectively, had viral shedding), and to prevent illness [48]. Intravenous zanamivir is active in a primate model of A(H5N1) virus infection [49], and the Southeast Asia Influenza Clinical Research Network [50] is developing a protocol to study it in patients with avian influenza A(H5N1) illness.

More recently, the NAI peramivir (BioCryst Pharmaceuticals) is currently in phase 2 trials for both intravenous and

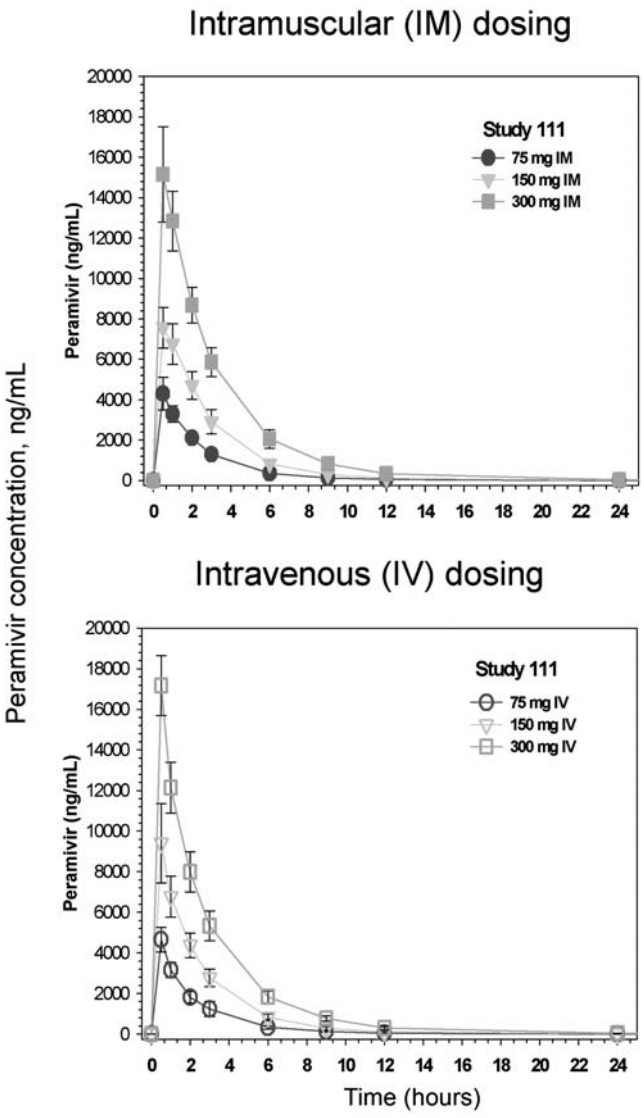

Figure 4. Plasma concentrations of peramivir after intramuscular and intravenous dosing in a phase 1 clinical trial. Adapted from [53], with permission.

intramuscular routes of administration. In vitro IC_{50} data for oseltamivir, zanamivir, and peramivir show that all 3 agents are potent inhibitors of influenza virus neuraminidases in the low nanomolar range (table 2) [34]. In an animal model of influenza A(H5N1) virus infection, mice receiving multiple oral doses of oseltamivir over 5 consecutive days or peramivir (administered as either a single intramuscular injection or as 5 intramuscular injections over 5 consecutive days), starting at 1 h after virus inoculation, were more likely to survive than were control mice injected with saline (table 4) [51]. The multiple-dose peramivir regimen was the only regimen that prevented paralysis by day 15 (table 4). However, intramuscular peramivir provided incomplete protection against neuroinvasive A(H5N1) disease in a second animal model. Among ferrets infected with 1 of 3 different doses of influenza A(H5N1) virus, overall survival improved with multiple doses of intramuscular peramivir (70%–86%), compared with survival among those given an intramuscular injection of saline (11%–43%); encephalitis was less common among peramivir recipients (32% [8/25]), compared with saline recipients (50% [13/26]); and paralysis developed in 8% (2/25) of animals that received treatment with multiple intramuscular doses of peramivir, compared with 42% (11/26) of animals given intramuscular doses of saline [51].

Peramivir has a prolonged plasma elimination half-life in humans and also appears to bind to the enzyme for a prolonged time [52]—2 factors that permit infrequent dosing regimens. In dose-ranging studies performed with healthy volunteers, the peak plasma concentrations for intramuscular and intravenous peramivir (10,000–20,000 ng/mL) are nearly 2 orders of magnitude higher than those achieved with standard doses of oral oseltamivir (~350 ng/mL; figure 4) [53]. It remains to be determined in clinical trials to what extent these higher levels in blood may provide greater antiviral efficacy and perhaps reduced frequency of resistance emergence. Future studies will need to determine whether such high plasma NAI levels will translate into greater clinical benefits for high-risk or hospitalized patients with influenza. A recent report indicated that a single 300- or 600-mg dose of intravenous peramivir was efficacious in the treatment of uncomplicated influenza in adult outpatients [54]

Long-acting NAIs. Biota Holdings of Australia and Sankyo Pharmaceuticals of Japan are codeveloping long-acting inhaled NAIs. CS-8958 (also known as R-118958) shows good activity in murine models of influenza treatment with once weekly dosing [55, 56]. Rennecke et al. [56] reported that, in healthy male subjects, R-118958 doses of 1, 2, 5, or 10 mg administered by inhalation did not result in the active metabolite being detectable in plasma, although it was detected in urine for up to 144 h after administration of the 5-mg and 10-mg doses. No serious adverse events or clinically significant changes in laboratory tests were noted. Available data suggest that CS-8958 may permit a more convenient topical dosing regimen, and the sponsors of a phase 2 study in Japan recently announced that

Table 5. In vitro anti-influenza activity and cellular cytotoxicity of T-705 versus ribavirin.

Antiviral agent	IC_{50}, μmol/L ± SD	
	Influenza A/PR/8/34(H1N1)	MDCK cells
T-705	1.0 ± 0.9	>6370
Ribavirin	31.6 ± 9.2	94.3 ± 47.6

NOTE. MDCK, Madin-Darby canine kidney. Adapted from [59], with permission from the American Society for Microbiology.

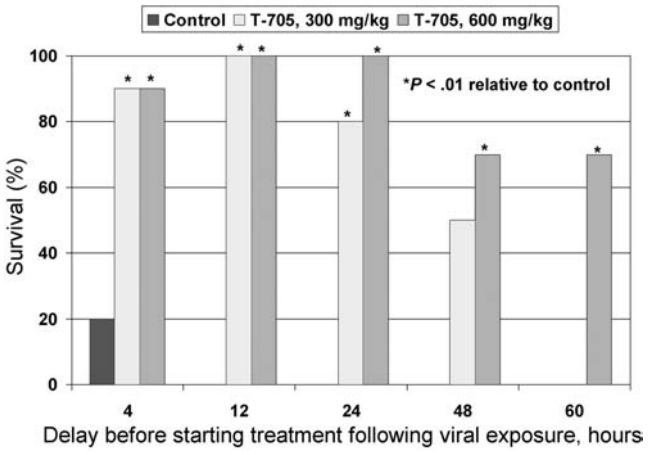

Figure 5. Effect of a single oral dose of T-705 on survival of mice exposed to lethal influenza A/Duck/N/1525/81(H5N1) virus. Adapted from [60], with permission from the American Society for Microbiology.

a single inhaled dose was found to be as effective as a standard 5-day course of oseltamivir in the treatment of uncomplicated influenza [57].

Polymerase inhibition. T-705 (Toyama Chemical) is not only active against all 3 influenza virus types (A, B, and C) but also has some activity against other RNA viruses, including some of the hemorrhagic fever viruses [58, 59]. T-705 undergoes ribosylation and then phosphorylation and thus functions like a nucleoside. Its primary mechanism of action is the inhibition of viral RNA polymerase. It appeared to show a more favorable therapeutic index than did ribavirin in preclinical tests of toxicity, including those done with human cells (table 5) [59], and has been shown to be active in murine models of influenza A(H5N1) virus infection (figure 5) [60]. These data demonstrated a survival benefit as late as 48 h after virus inoculation in a cohort given a 300-mg/kg/day dose and up to 60 h after virus inoculation in a cohort given the 600-mg/kg/day dose. Initial unpublished data on human pharmacology are encouraging with regard to oral absorption and tolerability, and phase 2 efficacy studies have been ongoing in Japan during the 2007–2008 influenza season.

Attachment inhibition. DAS181 (Fludase; NexBio) is a fusion construct that incorporates the sialidase from *Actinomyces viscosus,* a common oral bacterium linked to a human epithelium-anchoring domain; it can be mass produced in *Escherichia coli* [61]. The sialidase targets the viral attachment process, an early event in the replication of influenza virus. When this molecule is exposed to cells, it cleaves off the surface receptors on respiratory epithelium that are recognized by influenza hemagglutinin—both the α2,6-sialic acid–linked receptors to which human viruses attach and the α2,3-sialic acid–linked receptors to which avian viruses attach. DAS181 is inhibitory for a range of influenza A and B viruses, with in vitro EC_{90} values ranging from <1 to 56 nmol/L [61]. The epithelial tag on this molecule increases its activity by an order of magnitude (~5–30-fold) [61]. In vitro removal of receptors by DAS181 leads to a prolonged antiviral effect, although it is not clear whether this effect will translate into a less-frequent dosing regimen in the clinic. The molecule is not inhibitory for human cell growth.

Intranasal dosing has shown prophylactic and therapeutic activity in mice and antiviral effects with reduced inflammatory responses in ferrets [61]. Figure 6 illustrates the effect of DAS181 when administered intranasally in a rodent model of influenza [61]. In a murine model of highly pathogenic avian influenza using influenza A/Vietnam/1203/2004(H5N1)—a stringent test of an antiviral agent—DAS181 was active both prophylactically and therapeutically [62]. Administered prophylactically, a DAS181 dosage of 1 mg/kg/day protected 100% of mice from fatal disease and prevented viral dissemination to the brain. Therapeutically, antiviral effects and increased survival of mice exposed to an A(H5N1) virus challenge were noted when treatment began as late as 72 h after infection [62].

COMBINATION THERAPY

Combination therapy is not a new concept in the management of influenza. In fact, it initially was explored for the treatment of influenza in preclinical assays years before it became the standard of care in the management of HIV infection. Although a promising strategy, little data from controlled clinical trials of combination therapy for the treatment of influenza have been published. Several years ago, when circulating influenza A viruses were predictably susceptible to M2 inhibitors, the National Institute of Allergy and Infectious Diseases Collaborative Antiviral Study Group compared outcomes among hospitalized adults who received either nebulized zanamivir plus oral rimantadine or nebulized saline placebo plus oral rimantadine [63]. The small number of patients enrolled in this study

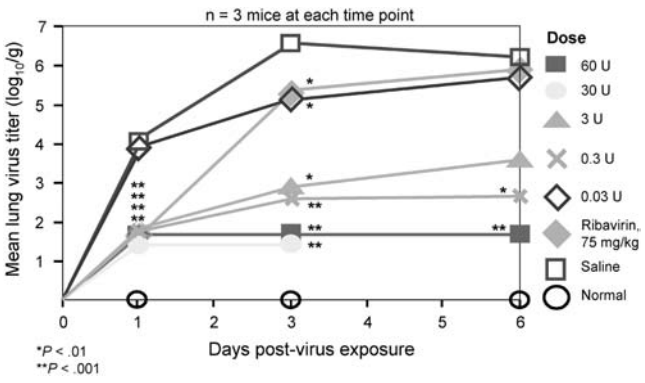

Figure 6. Effect of DAS181 on lung virus titers in mice. Adapted from [61], with permission from the American Society for Microbiology.

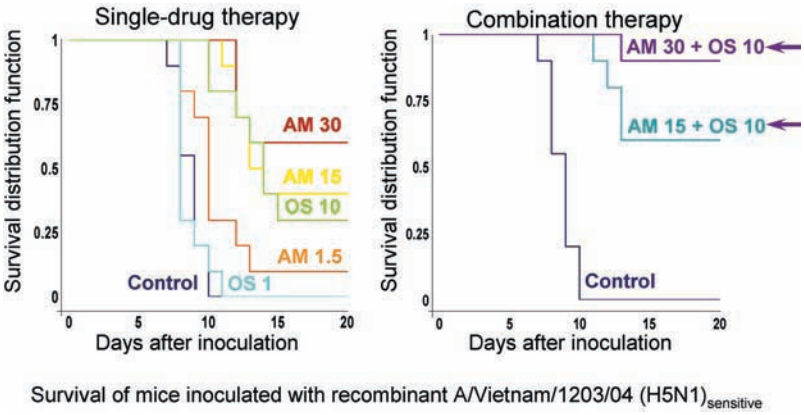

Figure 7. Effect of amantadine (AM)–oseltamivir (OS) combination therapy on survival of mice inoculated with influenza A(H5N1) virus. Mice received treatment, on the same schedule, with AM or OS at the doses indicated (in mg/kg/day), a combination of AM and OS, or saline placebo starting 24 h before virus inoculation. Adapted from [64].

limited its statistical power, but some encouraging trends were noted among patients who received combination therapy, compared with those who received rimantadine alone. Patients treated with zanamivir plus rimantadine demonstrated nonsignificant trends toward fewer days of viral shedding and reduced frequency of M2-inhibitor resistance. Patients assigned to combination therapy also were more likely to have either no cough or only a mild cough by the third day of treatment (15/16 [94%] vs. 11/20 [55%]; $P = .01$) [63]. No resistant variants were found in patients in the group receiving combination therapy, compared with 3 patients in the group receiving rimantadine alone.

M2 inhibitor–NAI combination therapy also has been investigated for the treatment of A(H5N1) disease. Figure 7 shows the effect of single drugs and combination therapy on survival among mice inoculated with an amantadine-sensitive strain of influenza A(H5N1) virus [64]. Although monotherapy showed dose-related effects, the combination of amantadine and oseltamivir at higher doses resulted in the highest level of survival and antiviral effects in this animal model. In contrast, no greater benefit was noted beyond the effects of oseltamivir alone when the infecting A(H5N1) virus was resistant to M2 inhibitors. This suggests that M2 inhibitor–NAI combination therapy is a potential treatment option for seriously ill patients if the influenza virus causing the infection is susceptible to M2 inhibitors. As previously noted, some A(H5N1) strains retain susceptibility to M2 inhibitors; consequently, the possibility of combination therapy has been suggested in recent WHO management guidelines [40].

Various other antiviral combinations have been studied or proposed, as shown in table 6 [65–73]. Further preclinical studies and eventually clinical trials will be invaluable for determining whether these new agents or suggested combinations will offer clinically meaningful benefits beyond those attained with current agents. However, until such clinical trial data are available, physicians must make difficult treatment decisions based on knowledge of patient characteristics and likely or actual antiviral susceptibility patterns. The following 2 cases are examples of such choices.

A kidney-transplant patient, aged 40 years, was admitted to the hospital with respiratory symptoms and influenza A virus infection in February 2007. In theory, this patient could have been treated with oseltamivir, zanamivir, or rimantadine, alone or in combination. Given the fact that the influenza virus infection was most likely due to an A(H3N2) virus that was resistant to the M2 inhibitors, there would be little point in

Table 6. Potential combination antiviral therapy for the treatment of influenza.

Evaluated in vitro
M2 inhibitor + IFN [65]
Evaluated in animal models
M2 inhibitor + thymosin-α1 + IFN [66]
M2 inhibitor + ribavirin [67]
M2 inhibitor + oseltamivir [64, 68]
NAI + ribavirin (or other transcriptase inhibitor) [69]
Evaluated in humans
Oral rimanatadine + nebulized zanamivir [63]
For future consideration
M2 inhibitor (with susceptible strains) + other NAI (e.g., intravenous zanamivir or peramivir)
Dual NAIs with differing cross-resistance patterns (e.g., zanamivir + oseltamivir)
Triple regimens with M2 inhibitor (with susceptible strains), NAI, ribavirin (or other transcriptase inhibitor), and/or possibly IFN-α [70]
Antivirals + antiviral antibodies [71]

NOTE. Antiviral combinations are also discussed in [44], [72], and [73]. NAI, neuraminidase inhibitor.

the use of rimantadine, since it would have increased the risk of toxicity with little chance of improving the clinical outcome. Combination therapy with oseltamivir and zanamivir would have offered the benefit of providing 2 NAIs with differing spectrums of action and sites of drug delivery. Unfortunately, the efficacy and tolerability of orally inhaled zanamivir for hospitalized patients or of this combination approach in relevant animal models have not been studied adequately. High-dose oral ribavirin [74] and intravenous ribavirin [75] are other possibilities but are investigational for the treatment of influenza. Thus, at that time, oral oseltamivir was the most practical treatment option.

By contrast, suppose that, during the course of an investigation into an avian influenza outbreak in Romania, a patient with confirmed poultry exposure is admitted to the hospital with a 5-day history of fever, cough, and now-increasing shortness of breath. Testing reveals influenza A(H5N1) virus infection, presumably owing to a clade 2.2 virus, and there is radiographic evidence of pneumonia. Currently, there is no evidence from randomized, controlled clinical trials to indicate selection of a specific antiviral intervention in this case. The updated WHO guidelines would recommend oseltamivir therapy but also would suggest consideration of alternative drug regimens, including combination treatment with an M2 inhibitor and oseltamivir, as well as doubling the oseltamivir dose and duration of therapy. Since almost all A(H5N1) viruses from clade 2.2 are susceptible to the M2 inhibitors, combination treatment with oseltamivir and rimantadine would be a reasonable approach. Given the low likelihood of survival of someone with pneumonic A(H5N1) disease, interventions that control replication as quickly as possible make sense. Hopefully, in future, parenteral agents, including specific monoclonal antibodies [76] or possibly convalescent plasma [77], will become available for study in such a patient.

CURRENT TRENDS IN ANTIVIRAL DRUGS FOR INFLUENZA TREATMENT

Numerous efforts are under way to develop new antiviral agents for influenza treatment that possess an improved spectrum of activity or better pharmacologic profiles, relative to current treatments. Some of the most desirable features of future antiviral agents for influenza treatment include greater potency that quickly curtails viral replication, longer plasma or pulmonary half-lives that permit fewer doses (or even a single therapeutic dose), reduction in the risk of development of resistant influenza virus strains, and completely new modes of antiviral action for use in combination therapies.

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